

**Figure 1**Sequence of the *Pichia methanolica* ketoreductase

ATG AAT TGG GAA AAA GTT CCA CAA GAA TTA TAC ACT  
 5 MET Asn Trp Glu Lys Val Pro Gln Glu Leu Tyr Thr  
  
 CGT TTG GGC TCT TCA GGT CTA CAA ATC TCC AAG ATT  
 Arg Leu Gly Ser Ser Gly Leu Gln Ile Ser Lys Ile  
  
 10 ATT GTT GGG TGT ATG TCA TTC GGT ACC AAA GCA TGG  
 Ile Val Gly Cys MET Ser Phe Gly Thr Lys Ala Trp  
  
 GGA GGT GAT TGG GTT TTG GAG GAT GAG GAT GAG ATC  
 Gly Gly Asp Trp Val Leu Glu Asp Glu Asp Glu Ile  
 15  
 TTT GCG ATT ATG AAA AAG GCT TAT GAT CAA GGT ATC  
 Phe Ala Ile MET Lys Lys Ala Tyr Asp Gln Gly Ile  
  
 AGA ACT TTT GAC ACT GCT GAC TCT TAT TCT AAT GGT  
 20 Arg Thr Phe Asp Thr Ala Asp Ser Tyr Ser Asn Gly  
  
 GTT TCT GAA AGA CTC TTA GGT AAA TTC ATT AGA AAG  
 Val Ser Glu Arg Leu Leu Gly Lys Phe Ile Arg Lys  
  
 25 TAC AAC ATT GAT AGA TCT AAG CTT GTT ATT TTG ACT  
 Tyr Asn Ile Asp Arg Ser Lys Leu Val Ile Leu Thr  
  
 AAG GTT TTT TTC CCA GCT CCT GAA GAA TAT GAG TCG  
 Lys Val Phe Phe Pro Ala Pro Glu Glu Tyr Glu Ser  
 30  
 TTT AGC TTC TTT AAT CAT AAT TTC CCT GGT CAC GAG  
 Phe Ser Phe Phe Asn His Asn Phe Pro Gly His Glu

TTG GTC AAC AGA AGT GGC TTA TCG AGA AAA CAT ATT  
Leu Val Asn Arg Ser Gly Leu Ser Arg Lys His Ile

TTG GAC TCT GCT GCT GCC TCT GTT GAG AGA TTA GGC  
5 Leu Asp Ser Ala Ala Ala Ser Val Glu Arg Leu Gly

ACC TAT ATC GAT GTA CTA CAA ATT CAT AGA TAT GAT  
Thr Tyr Ile Asp Val Leu Gln Ile His Arg Tyr Asp

10 CCA AAT ACC CCT GCT GAA GAA ACC ATG GAA GCT TTG  
Pro Asn Thr Pro Ala Glu Glu Thr MET Glu Ala Leu

AAT GAT TGT ATT AAA CAA GGT TTA ACC AGA TAC ATT  
Asn Asp Cys Ile Lys Gln Gly Leu Thr Arg Tyr Ile

15

GGA GCA TCT ACC ATG AGA GCC TAT CAA TTC ATC AAG  
Gly Ala Ser Thr MET Arg Ala Tyr Gln Phe Ile Lys

TAT CAA AAC GTT GCT GAG AAA CAT GGG TGG GCA AAG  
20 Tyr Gln Asn Val Ala Glu Lys His Gly Trp Ala Lys

TTC ATC TCG ATG CAA AGC TAC TAC AGT TTA CTT TAC  
Phe Ile Ser MET Gln Ser Tyr Tyr Ser Leu Leu Tyr

25 CGT GAA GAA GAA GCA GAA CTA ATT GCA TAC TGT AAT  
Arg Glu Glu Glu Ala Glu Leu Ile Ala Tyr Cys Asn

GAA ACT GGT GTT GGG TTA ATC CCA TGG TCA CCA AAC  
Glu Thr Gly Val Gly Leu Ile Pro Trp Ser Pro Asn

30

GCT GGT GGA TTC TTA ACC AGA CCA GTA TCC AAG CAA  
Ala Gly Gly Phe Leu Thr Arg Pro Val Ser Lys Gln

GAC ACT GCG AGA AGT GCA AGT GGG GCT GCT GCG TTA  
 Asp Thr Ala Arg Ser Ala Ser Gly Ala Ala Ala Leu

TAT GGT CTA GAA CCT TTC AGT GAG GCT GAT AAG GCT  
 5 Tyr Gly Leu Glu Pro Phe Ser Glu Ala Asp Lys Ala

ATT ATT GAC AGG GTT GAA GAG TTA TCA AAG AAA AAG  
 Ile Ile Asp Arg Val Glu Glu Leu Ser Lys Lys Lys

10 GGA GTT TCT ATG GCT AGT GTC GCT TTA GCT TGG GTT  
 Gly Val Ser MET Ala Ser Val Ala Leu Ala Trp Val

ATT AGT AAG AAC AGT TGG CCA ATT ATT GGT TTC AGT  
 Ile Ser Lys Asn Ser Trp Pro Ile Ile Gly Phe Ser  
 15

AAG CCT GGA AGG GTT GAT GAT GCT TTA GAT GGT TTC  
 Lys Pro Gly Arg Val Asp Asp Ala Leu Asp Gly Phe

AAG TTG AAG CTA ACC GAA GAG GAC ATC AAA TTC TTA  
 20 Lys Leu Lys Leu Thr Glu Glu Asp Ile Lys Phe Leu

GAA GAG CCT TAT GTT CCA AAA CCT TTG CCT CGC TTA  
 Glu Glu Pro Tyr Val Pro Lys Pro Leu Pro Arg Leu

25 TAT TCT GTA ATT TTA TAA  
 Tyr Ser Val Ile Leu STP

### Example 9

*Reduction of Keto Methyl Ester (V) by Recombinant Escherichia coli Expressing Keto-reductase from Pichia methanolica:*

- 5            Keto-reductase gene from *Pichia methanolica* was cloned and overexpressed in *Escherichia coli*. Cells of *Escherichia coli* (expressing keto-reductase) were grown in a 15-L and 250-L fermentor in medium 5. Induction of Keto-reductase in *Escherichia coli* was carried out at optical cell density (OD) of culture was reached to 3.0 by the addition of 0.25 mM isopropyl- $\beta$ -thiogalactoside (IPTG) as an inducer.
- 10        Cells were harvested after 30 hours growth in a fermentor after addition of IPTG. Cells were used to catalyze the bioconversion of keto methyl ester (substrate V) to the corresponding (S)-hydroxy methyl ester (product VI) by cell suspensions.

- The substrate and the product for this Example were as described in Example
- 15        3. Cells of *Escherichia coli* expressing Ketoreductase enzyme were suspended in 1-L of 100 mM phosphate buffer pH 7.0 at a 10% (W/V) cell concentration. Cell suspensions were supplemented with 100  $\mu$ M nicotinamide adenine dinucleotide phosphate (NADP), 1 mM phenylmethane sulfonyl fluoride (PMSF), 50 grams glucose, 3400 units glucose dehydrogenase, and 4.5 grams substrate (keto methyl
- 20        ester, Formula V). Biotransformation was carried out at 500 RPM and at 28°C temperature. Substrate V and product VI concentrations and enantiomeric excess of product VI were determined by HPLC analysis as described in the example 3. The reaction was completed in 20 hours with a reaction yield of product (hydroxymethyl ester, Formula VI) of 95%. The enantiomeric excess of 99.9% was obtained for
- 25        product VI.